Antibacterial Agents

This invention relates to novel hydroxamic acid and N-formyl hydroxylamine derivatives having antibacterial activity, to methods of treatment using such compounds, and to pharmaceutical and veterinary compositions comprising such compounds.

Background to the Invention

Many classes of antibacterial agents are known, including the penicillins and cephalosporins, tetracyclines, sulfonamides, monobactams, fluoroquinolones and quinolones, aminoglycosides, glycopeptides, macrolides, polymyxins, lincosamides, trimethoprim and chloramphenicol. The fundamental mechanisms of action of these antibacterial classes vary.

Bacterial resistance to many known antibacterials is a growing problem. Accordingly there is a continuing need in the art for alternative antibacterial agents, especially those which have mechanisms of action fundamentally different from the known classes.

Amongst the Gram-positive pathogens, such as Staphylococci, Streptococci, Mycobacteria and Enterococci, resistant strains have evolved/arisen which makes them particularly difficult to eradicate. Examples of such strains are methicillin resistant *Staphylococcus aureus* (MRSA), methicillin resistant coagulase negative Staphylococci (MRCNS), penicillin resistant *Streptococcus pneumoniae* and multiply resistant *Enterococcus faecium.*

Pathogenic bacteria are often resistant to the aminoglycoside, β-lactam (penicillins and cephalosporins), and chloramphenicol types of antibiotic. This resistance involves the enzymatic inactivation of the antibiotic by hydrolysis or by formation of inactive derivatives. The β-lactam (penicillin and cephalosporin) family of antibiotics are characterised by the presence of a β-lactam ring structure. Resistance to this

family of antibiotics in clinical isolates is most commonly due to the production of a "penicillinase" (β-lactamase) enzyme by the resistant bacterium which hydrolyses the β-lactam ring thus eliminating its antibacterial activity.

Recently there has been an emergence of vancomycin-resistant strains of enterococci (Woodford N. 1998 Glycopeptide-resistant enterococci: a decade of experience. Journal of Medical Microbiology. 47(10):849-62). Vancomycin-resistant enterococci are particularly hazardous in that they are frequent causes of hospital based infections and are inherently resistant to most antibiotics. Vancomycin works by binding to the terminal D-Ala-D-Ala residues of the cell wall peptidioglycan precursor. The high-level resistance to vancomycin is known as VanA and is conferred by a genes located on a transposable element which alter the terminal residues to D-Ala-D-lac thus reducing the affinity for vancomycin.

In view of the rapid emergence of multidrug-resistant bacteria, the development of antibacterial agents with novel modes of action that are effective against the growing number of resistant bacteria, particularly the vancomycin resistant enterococci and β-lactam antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus,* is of utmost importance.

Brief Description of the Invention

This invention is based on the finding that certain hydroxamic acid and N-formyl hydroxylamine derivatives have antibacterial activity, and makes available a new group of antibacterial agents. It has been found that the compounds with which this invention is concerned are antibacterial with respect to a range of bacteria, with potency against Gram-positive organisms generally being greater than against Gram-negatives. Many of the compounds of the invention show activity against bacteria responsible for respiratory infections, such as *Streptococcus pneumoniae* and *Haemophilus influenzae.*

Although it may be of interest to establish the mechanism of action of the

compounds with which the invention is concerned, it is their ability to inhibit bacterial growth that makes them useful. However, it is presently believed that their antibacterial activity is due, at least in part, to intracellular inhibition of bacterial polypeptide deformylase (PDF; EC 3.5.1.31).

All ribosome-mediated synthesis of proteins starts with a methionine residue. In prokaryotes the methionyl moiety carried by the initiator tRNA is N-formylated prior to its incorporation into a polypeptide. Consequently, N-formylmethionine is always present at the N-terminus of a nascent bacterial polypeptide. However, most mature proteins do not retain the N-formyl group or the terminal methionine residue. Deformylation is required prior to methionine removal, since methionine aminopeptidase does not recognise peptides with an N-terminal formylmethionine residue (Solbiati et al., J. Mol. Biol. 290:607-614, 1999). Deformylation is, therefore, a crucial step in bacterial protein biosynthesis and the enzyme responsible, PDF, is essential for normal bacterial growth. Although the gene encoding PDF (*def*) is present in all pathogenic bacteria for which sequences are known (Meinnel et al., J. Mol. Biol, 266:939-49, 1997), it has no eukaryotic counterpart, making it an attractive target for antibacterial chemotherapy.

The isolation and characterisation of PDF has been facilitated by an understanding of the importance of the metal ion in the active site (Groche et al., Biophys. Biochem. Res. Commun., 246:324-6, 1998). The Fe^{2+} form is highly active *in vivo* but is unstable when isolated due to oxidative degradation (Rajagopalan et al., J. Biol. Chem. 273:22305-10, 1998). The Ni^{2+} form of the enzyme has specific activity comparable with the ferrous enzyme but is oxygen-insensitive (Ragusa et al., J. Mol. Biol. 1998, 280:515-23, 1998). The Zn^{2+} enzyme is also stable but is almost devoid of catalytic activity (Rajagopalan et al., J. Am. Chem. Soc. 119:12418-12419, 1997).

Several X-ray crystal structures and NMR structures of *E. coli* PDF, with or without bound inhibitors, have been published (Chan et al., Biochemistry 36:13904-9, 1997; Becker et al., Nature Struct. Biol. 5:1053-8, 1998; Becker et al., J. Biol. Chem.

273:11413-6, 1998; Hao et al., Biochemistry, 38:4712-9, 1999; Dardel et al., J. Mol. Biol. 280:501-13, 1998; O'Connell et al., J. Biomol. NMR, 13:311-24, 1999), indicating similarities in active site geometry to metalloproteinases such as thermolysin and the metzincins.

Recently the substrate specificity of PDF has been extensively studied (Ragusa et al., J. Mol. Biol. 289:1445-57, 1999; Hu et al., Biochemistry 38:643-50, 1999; Meinnel et al., Biochemistry, 38:4287-95, 1999). These authors conclude that an unbranched hydrophobic chain is preferred at P1', while a wide variety of P2' substituents are acceptable and an aromatic substituent may be advantageous at the P3' position. There have also been reports that small peptidic compounds containing an H-phosphonate (Hu et al., Bioorg. Med. Chem. Lett., 8:2479-82, 1998) or thiol (Meinnel et al., Biochemistry, 38:4287-95, 1999) metal binding group are micromolar inhibitors of PDF. Peptide aldehydes such as calpeptin (N-Cbz-Leu-norleucinal) have also been shown to inhibit PDF (Durand et al., Arch. Biochem. Biophys., 367:297-302, 1999). However, the identity of the metal binding group and its spacing from the rest of the molecule ("recognition fragment") has not been studied extensively. Furthermore, non-peptidic PDF inhibitors, which may be desirable from the point of view of bacterial cell wall permeability or oral bioavailability in the host species, have not been identified.

Related Prior Art

Certain N-formyl hydroxylamine derivatives have previously been claimed in the patent publications listed below, although very few examples of such compounds have been specifically made and described:

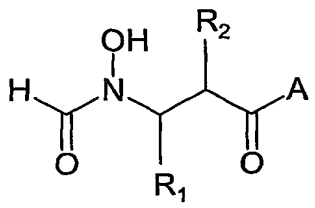
EP-B-0236872	(Roche)
WO 92/09563	(Glycomed)
WO 92/04735	(Syntex)
WO 95/19965	(Glycomed)
WO 95/22966	(Sanofi Winthrop)

WO 95/33709	(Roche)
WO 96/23791	(Syntex)
WO 96/16027	(Syntex/Agouron)
WO 97/03783	(British Biotech)
WO 97/18207	(DuPont Merck)
WO 98/38179	(GlaxoWellcome)
WO 98/47863	(Labs Jaques Logeais)

The pharmaceutical utility ascribed to the N-formyl hydroxylamine derivatives in those publications is the ability to inhibit matrix metalloproteinases (MMPs) and in some cases release of tumour necrosis factor (TNF), and hence the treatment of diseases or conditions mediated by those enzymes, such as cancer and rheumatoid arthritis.

In addition to these, US-A-4,738,803 (Roques et al.) also discloses N-formyl hydroxylamine derivatives, however, these compounds are disclosed as enkephalinase inhibitors and are proposed for use as antidepressants and hypotensive agents. Also, WO 97/38705 (Bristol-Myers Squibb) discloses certain N-formyl hydroxylamine derivatives as enkephalinase and angiotensin converting enzyme inhibitors.

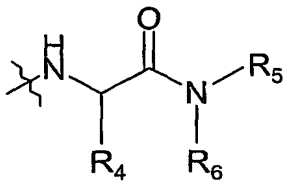
Our copending International Patent Application No. WO 99/39704 describes and claims, *inter alia*, the use of a compound of formula (I) or a pharmaceutically or veterinarily acceptable salt thereof in the preparation of an antibacterial composition:



(I)

wherein R_1 represents hydrogen, C_1-C_6 alkyl or C_1-C_6 alkyl substituted by one or more halogen atoms; R_2 represents a substituted or unsubstituted C_1-C_6 alkyl,

cycloalkyl(C_1-C_6 alkyl)- or aryl(C_1-C_6 alkyl)- group; and A represents a group of formula (IA), or (IB):



(IA)

(IB)

wherein R_4 represents the side chain of a natural or non-natural alpha amino acid, and R_5 and R_6 when taken together with the nitrogen atom to which they are attached form an optionally substituted saturated heterocyclic ring of 3 to 8 atoms which ring is optionally fused to a carbocyclic or second heterocyclic ring.

Very many hydroxamic acid derivatives are known. Many have been disclosed as having matrix metalloproteinase (MMP) inhibitory activity, and thus to be potentially useful for the treatment of diseases mediated by MMPs, for example cancer, arthritides, and conditions involving tissue remodeling such as wound healing, and restenosis. In addition our International Patent Application No. WO 99/59568 describes the use of analogues of the N-formylhydroxylamine derivatives of WO 99/39704 (wherein the N-formylhydroxylamine group is replaced by a hydroxamic acid group) in the preparation of an antibacterial composition.

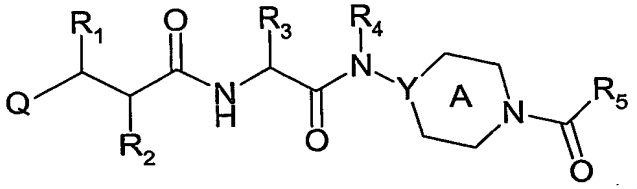
Brief Description of the Invention

This invention relates to a group of antibacterially active hydroxamic acid and and N-formyl hydroxylamine compounds which differ in structure from those of International Patent Applications Nos. WO 99/59568 and WO 99/39704, principally in the nature of the -NR_5R_6 group (see formulae (I), (IA) above and the hydroxamic acid analogues thereof). In those applications, R_5 may be C_1-C_6 alkyl and R_6 may be an optionally substituted heterocuclic ring. The term "optionally substituted" as used in

relation to the saturated heterocyclic ring R_6 is defined as meaning certain specific substituents. In the present compounds, the group R_5 is also C_1-C_6 alkyl and R_6 is also substituted heterocyclic ring, namely piperidinyl or piperazinyl, but the substituents are different from those permitted by WO 99/59568 and WO 99/39704. The group $-NR_5R_6$ of the N-formyl hydroxylamines and hydroxamic acids of the invention is also believed to distinguish the present compounds from those known in the MMP, TNF, ACE, and enkephalinase inhibitor art.

Detailed description of the invention

The present invention provides a compound of formula (II), or a pharmaceutically or veterinarily acceptable salt, hydrate or solvate thereof



(II)

wherein

Q represents a radical of formula -N(OH)CH(=O) or formula -C(=O)NH(OH);

R_1 represents hydrogen, methyl or trifluoromethyl, or, except when Z is a radical of formula -N(OH)CH(=O), a hydroxy, halo or amino group;

R_2 represents a group R_{10}-$(V)_n$-$(ALK)_m$- wherein

R_{10} represents hydrogen, or a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, cycloalkyl, aryl, or heterocyclyl group, any of which may be unsubstituted or substituted by (C_1-C_6)alkyl, (C_1-C_6)alkoxy, hydroxy, mercapto, (C_1-C_6)alkylthio, amino, halo (including fluoro, chloro, bromo and iodo), trifluoromethyl, cyano, nitro, oxo, -COOH, $-CONH_2$, $-COOR^A$, $-NHCOR^A$, $-CONHR^A$, $-NHR^A$, $-NR^AR^B$, or $-CONR^AR^B$ wherein R^A and R^B are independently a (C_1-C_6)alkyl group and

ALK represents a straight or branched divalent C_1-C_6 alkylene, C_2-C_6 alkenylene, or C_2-C_6 alkynylene radical, and may be interrupted by one or more non-adjacent -NH-, -O- or -S- linkages,

V represents -NH-, -O- or -S-, and

m and n are independently 0 or 1;

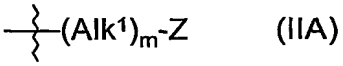
R_3 represents the side chain of a natural or non-natural alpha amino acid;

R_4 represents hydrogen or C_1-C_3 alkyl;

Y represents N or CH;

ring A is optionally substituted on one or more ring carbon atoms by C_1-C_3 alkyl, C_1-C_3 alkoxy, or halo; and

R_5 represents a group (IIA),



(IIA)

wherein

m is 0 or 1;

Alk^1 represents a divalent C_1-C_3 alkylene radical;

Z represents hydrogen or cycloalkyl, phenyl or heterocyclic which is optionally substituted by

$(C_1$-$C_6)$alkyl,

phenyl

monocyclic 5 or 6-membered heterocyclic,
benzyl,
phenoxy, or (C_1-C_6)alkoxy,
phenylthio or (C_1-C_6)alkylthio, any of which is in turn optionally substituted by:
hydroxy or mercapto,
trifluoromethyl,
oxo,
nitro,
cyano (-CN)
bromo, chloro, fluoro, or iodo
-COOH, or -$COOR^A$,
-$CONH_2$, -$CONHR^A$, or -$CONR^AR^B$
-COR^A, -SO_2R^A,
-$NHCOR^A$,
-NH_2, -NHR^A, or -NR^AR^B,
wherein R^A and R^B are independently a (C_1-C_6) alkyl group, or R^A and R^B taken together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring which may besubstituted by (C_1C_3)alkyl, hydroxy, or hydroxy(C_1-C_3)alkyl.

In a subset of the compounds of the invention, Z represents a cycloalkyl, phenyl or monocyclic heterocyclic ring, which is optionally substituted by
(C_1-C_6)alkyl, (C_2-C_6)alkenyl, or (C_2-C_6)alkynyl,
phenyl, or halophenyl,
trifluoromethyl,
monocyclic 5 or 6-membered hetrocyclic,
benzyl, or halophenylmethyl,
hydroxy, phenoxy, (C_1-C_6)alkoxy, or hydroxy(C_1-C_6)alkyl,
mercapto, (C_1-C_6)alkylthio or mercapto(C_1-C_6)alkyl,

oxo,

nitro,

cyano (-CN)

bromo, chloro, fluoro, or iodo

-COOH, or -$COOR^A$,

-$CONH_2$, -$CONHR^A$, or -$CONR^AR^B$

-COR^A, -SO_2R^A,

-$NHCOR^A$,

-NH_2, -NHR^A, or -NR^AR^B,

wherein R^A and R^B are independently a (C_1-C_6) alkyl group, or R^A and R^B taken together with the nitrogen atom to which they are attached form a 5- or 6-membered heterocyclic ring which may be substituted by (C_1C_3)alkyl, hydroxy, or hydroxy(C_1-C_3)alkyl.

In another aspect, the invention provides a method for the treatment of bacterial infections in humans and non-human mammals, which comprises administering to a subject suffering such infection an antibacterially effective dose of a compound of formula (II) as defined above.

In a further aspect of the invention there is provided a method for the treatment of bacterial contamination by applying an antibacterially effective amount of a compound of formula (II) as defined above to the site of contamination.

The compounds of formula (II) as defined above may be used as component(s) of antibacterial cleaning or disinfecting materials.

On the hypothesis that the compounds (II) act by inhibition of intracellular PDF, the most potent antibacterial effect may be achieved by using compounds which

efficiently pass through the bacterial cell wall. Thus, compounds which are highly active as inhibitors of PDF in vitro and which penetrate bacterial cells are preferred for use in accordance with the invention. It is to be expected that the antibacterial potency of compounds which are potent inhibitors of the PDF enzyme in vitro, but are poorly cell penetrant, may be improved by their use in the form of a prodrug, ie a structurally modified analogue which is converted to the parent molecule of formula (II), for example by enzymic action, after it has passed through the bacterial cell wall.

As used herein the term "(C_1-C_6)alkyl" means a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms, including for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl and n-hexyl.

As used herein the term "divalent (C_1-C_3)alkylene radical" means a saturated hydrocarbon chain having from 1 to 3 carbon atoms and two unsatisfied valencies.

As used herein the term "(C_2-C_6)alkenyl" means a straight or branched chain alkenyl moiety having from 2 to 6 carbon atoms having at least one double bond of either E or Z stereochemistry where applicable. The term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

As used herein the term "C_2-C_6 alkynyl" refers to straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl and 5-hexynyl.

As used herein the term "cycloalkyl" means a saturated alicyclic moiety having from 3-8 carbon atoms and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

As used herein the term "heteroaryl" refers to a 5- or 6- membered aromatic ring

containing one or more heteroatoms;. Illustrative of such groups are thienyl, furyl, pyrrolyl, imidazolyl, benzimidazolyl, thiazolyl, pyrazolyl, isoxazolyl, isothiazolyl, triazolyl, thiadiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl.

As used herein the unqualified term "heterocyclyl" or "heterocyclic" includes "heteroaryl" as defined above, and in particular means a 5-7 membered aromatic or non-aromatic heterocyclic ring containing one or more heteroatoms selected from S, N and O, including for example, pyrrolyl, furanyl, thienyl, piperidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, morpholinyl, benzofuranyl, pyranyl, isoxazolyl, benzimidazolyl, methylenedioxyphenyl, maleimido and succinimido groups.

As used herein the terms "side chain of a natural alpha-amino acid" and Aside chain of a non-natural alpha-amino acid≅ mean the group R^x in respectively a natural and non-natural amino acid of formula NH_2-CH(R^x)-COOH.

Examples of side chains of natural alpha amino acids include those of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, histidine, 5-hydroxylysine, 4-hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, α-aminoadipic acid, α-amino-n-butyric acid, 3,4-dihydroxyphenylalanine, homoserine, α-methylserine, ornithine, pipecolic acid, and thyroxine.

In natural alpha-amino acid side chains which contain functional substituents, for example amino, carboxyl, hydroxy, mercapto, guanidyl, imidazolyl, or indolyl groups as in arginine, lysine, glutamic acid, aspartic acid, tryptophan, histidine, serine, threonine, tyrosine, and cysteine, such functional substituents may optionally be protected.

Likewise, in the side chains of non-natural alpha amino acids which contain functional substituents, for example amino, carboxyl, hydroxy, mercapto, guanidyl, imidazolyl, or indolyl groups, such functional substituents may optionally be protected.

The term "protected" when used in relation to a functional substituent in a side chain of a natural or non-natural alpha-amino acid means a derivative of such a substituent which is substantially non-functional. The widely used handbook by T. W. Greene and P. G. Wuts "Protective Groups in Organic Synthesis" Second Edition, Wiley, New York, 1991 reviews the subject. For example, carboxyl groups may be esterified (for example as a C_1-C_6 alkyl ester), amino groups may be converted to amides (for example as a $NHCOC_1$-C_6 alkyl amide) or carbamates (for example as an $NHC(=O)OC_1$-C_6 alkyl or $NHC(=O)OCH_2Ph$ carbamate), hydroxyl groups may be converted to ethers (for example an OC_1-C_6 alkyl or a $O(C_1$-C_6 alkyl)phenyl ether) or esters (for example a $OC(=O)C_1$-C_6 alkyl ester) and thiol groups may be converted to thioethers (for example a tert-butyl or benzyl thioether) or thioesters (for example a $SC(=O)C_1$-C_6 alkyl thioester).

There are several actual or potential chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetric carbon atoms gives rise to a number of diastereoisomers with R or S stereochemistry at each chiral centre. The invention includes all such diastereoisomers and mixtures thereof. Currently, the preferred stereoconfiguration of the carbon atom carrying the R_2 group is R; that of the carbon atom carrying the R_4 group (when asymmetric) is S; and that of the carbon atom carrying the R_1 group (when asymmetric) is R.

In the compounds of the invention:

When Q is a radical of formula -N(OH)CH(=O), R_1 may be, for example, hydrogen, methyl, or trifuoromethyl. Hydrogen is currently preferred.

When Q is a radical of formula -C(=O)NH(OH), R_1 may be, for example, hydrogen, methyl, trifluoromethyl, hydroxy, halo or amino. Again hydrogen is currently preferred.

R_2 may be, for example:

optionally substituted C_1-C_8 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl or cycloalkyl;

phenyl(C_1-C_6 alkyl)-, phenyl(C_3-C_6 alkenyl)- or phenyl(C_3-C_6 alkynyl)- optionally substituted in the phenyl ring;

cycloalkyl(C_1-C_6 alkyl)- for example cycloalkylmethyl, cycloalkyl(C_3-C_6 alkenyl)- or cycloalkyl(C_3-C_6 alkynyl)-, optionally substituted in the cycloalkyl ring;

heterocyclyl(C_1-C_6 alkyl)-, heterocyclyl(C_3-C_6 alkenyl)- or heterocyclyl(C_3-C_6 alkynyl)- optionally substituted in the heterocyclyl ring; or

(C_1-C_3)alkyl-S-(C_1-C_3)alkyl-, or (C_1-C_3)alkyl-O-(C_1-C_3)alkyl-.

Specific examples of R_2 groups include

methyl, ethyl, n- and iso-propyl, n- and iso-butyl, n-pentyl, iso-pentyl 3-methyl-but-1-yl, n-hexyl, n-heptyl, n-acetyl, n-octyl, methylsulfanylethyl, ethylsulfanylmethyl, 2-methoxyethyl, 2-ethoxyethyl, 2-ethoxymethyl, 3-hydroxypropyl, allyl, 3-phenylprop-3-en-1-yl, prop-2-yn-1-yl, 3-phenylprop-2-yn-1-yl, 3-(2-chlorophenyl)prop-2-yn-1-yl, but-2-yn-1-yl, cyclopentyl, cyclohexyl, cyclopentylmethyl, cyclopentylethyl, cyclopentylpropyl, cyclohexylmethyl, cyclohexylethyl, cyclohexylpropyl, furan-2-ylmethyl, furan-3-methyl, tetrahydrofuran-2-ylmethyl, tetrahydrofuran-2-ylmethyl, piperidinylmethyl, phenylpropyl, 4-chlorophenylpropyl, 4-methylphenylpropyl, 4-methoxyphenylpropyl, benzyl, 4-chlorobenzyl, 4-methylbenzyl, and 4-

methoxybenzyl.

Presently preferred groups at R_2 are $(C_1$-$C_6)$alkyl-, cycloalkylmethyl-, $(C_1$-$C_3)$alkyl-S-$(C_1$-$C_3)$alkyl-, or $(C_1$-$C_3)$alkyl-O-$(C_1$-$C_3)$alkyl-, especially n-propyl, n-butyl, n-pentyl, cyclopentylmethyl, cyclopentylethyl, cyclohexylmethyl or cyclohexylethyl.

R_3 may be, for example

the characterising group of a natural α amino acid, for example benzyl, or 4-methoxyphenylmethyl, in which any functional group may be protected, any amino group may be acylated and any carboxyl group present may be amidated; or

a group $-[Alk]_nR_9$ where Alk is a $(C_1$-$C_6)$alkylene or $(C_2$-$C_6)$alkenylene group optionally interrupted by one or more -O-, or -S- atoms or $-N(R_{12})$- groups [where R_{12} is a hydrogen atom or a $(C_1$-$C_6)$alkyl group], n is 0 or 1, and R_9 is hydrogen or an optionally substituted phenyl, aryl, heterocyclyl, cycloalkyl or cycloalkenyl group or (only when n is 1) R_9 may additionally be hydroxy, mercapto, $(C_1$-$C_6)$alkylthio, amino, halo, trifluoromethyl, nitro, -COOH, $-CONH_2$, $-COOR^A$, $-NHCOR^A$, $-CONHR^A$, $-NHR^A$, $-NR^AR^B$, or $-CONR^AR^B$ wherein R^A and R^B are independently a $(C_1$-$C_6)$alkyl group; or

a benzyl group substituted in the phenyl ring by a group of formula $-OCH_2COR_8$ where R_8 is hydroxyl, amino, $(C_1$-$C_6)$alkoxy, phenyl$(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkylamino, di$((C_1$-$C_6)$alkyl)amino, phenyl$(C_1$-$C_6)$alkylamino; or

a heterocyclic$(C_1$-$C_6)$alkyl group, either being unsubstituted or mono- or di-substituted in the heterocyclic ring with halo, nitro, carboxy, $(C_1$-$C_6)$alkoxy, cyano, $(C_1$-$C_6)$alkanoyl, trifluoromethyl $(C_1$-$C_6)$alkyl, hydroxy, formyl, amino, $(C_1$-$C_6)$alkylamino, di-$(C_1$-$C_6)$alkylamino, mercapto, $(C_1$-$C_6)$alkylthio, hydroxy$(C_1$-$C_6)$alkyl, mercapto$(C_1$-$C_6)$alkyl or $(C_1$-$C_6)$alkylphenylmethyl; or

a group $-CR_aR_bR_c$ in which:

each of R_a, R_b and R_c is independently hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, phenyl(C_1-C_6)alkyl, (C_3-C_8)cycloalkyl; or

R_c is hydrogen and R_a and R_b are independently phenyl or heteroaryl such as pyridyl; or

R_c is hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, phenyl(C_1-C_6)alkyl, or (C_3-C_8)cycloalkyl, and R_a and R_b together with the carbon atom to which they are attached form a 3 to 8 membered cycloalkyl or a 5- to 6-membered heterocyclic ring; or

R_a, R_b and R_c together with the carbon atom to which they are attached form a tricyclic ring (for example adamantyl); or

R_a and R_b are each independently (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, phenyl(C_1-C_6)alkyl, or a group as defined for R_c below other than hydrogen, or R_a and R_b together with the carbon atom to which they are attached form a cycloalkyl or heterocyclic ring, and R_c is hydrogen, -OH, -SH, halogen, -CN, $-CO_2H$, (C_1-C_4)perfluoroalkyl, $-CH_2OH$, $-CO_2(C_1-C_6)$alkyl, $-O(C_1-C_6)$alkyl, $-O(C_2-C_6)$alkenyl, $-S(C_1-C_6)$alkyl, $-SO(C_1-C_6)$alkyl, $-SO_2(C_1-C_6)$ alkyl, $-S(C_2-C_6)$alkenyl, $-SO(C_2-C_6)$alkenyl, $-SO_2(C_2-C_6)$alkenyl or a group -Q-W wherein Q represents a bond or -O-, -S-, -SO- or $-SO_2-$ and W represents a phenyl, phenylalkyl, (C_3-C_8)cycloalkyl, (C_3-C_8)cycloalkylalkyl, (C_4-C_8)cycloalkenyl, (C_4-C_8)cycloalkenylalkyl, heteroaryl or heteroarylalkyl group, which group W may optionally be substituted by one or more substituents independently selected from, hydroxyl, halogen, -CN, $-CO_2H$, $-CO_2(C_1-C_6)$alkyl, $-CONH_2$, $-CONH(C_1-C_6)$alkyl, $-CONH(C_1-C_6\text{alkyl})_2$, -CHO, $-CH_2OH$, (C_1-C_4)perfluoroalkyl, $-O(C_1-C_6)$alkyl, $-S(C_1-$

C_6)alkyl, -SO(C_1-C_6)alkyl, -SO_2(C_1-C_6)alkyl, -NO_2, -NH_2, -NH(C_1-C_6)alkyl, -N((C_1-C_6)alkyl)$_2$, -NHCO(C_1-C_6)alkyl, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, (C_3-C_8)cycloalkyl, (C_4-C_8)cycloalkenyl, phenyl or benzyl.

Examples of particular R_3 groups include methyl, ethyl, benzyl, 4-chlorobenzyl, 4-hydroxybenzyl, phenyl, cyclohexyl, cyclohexylmethyl, pyridin-3-ylmethyl, tert-butoxymethyl, naphthylmethyl, iso-butyl, sec-butyl, tert-butyl, 1-benzylthio-1-methylethyl, 1-methylthio-1-methylethyl, 1-mercapto-1-methylethyl, 1-methoxy-1-methylethyl, 1-hydroxy-1-methylethyl, 1-fluoro-1-methylethyl, hydroxymethyl, 2-hydroxethyl, 2-carboxyethyl, 2-methylcarbamoylethyl, 2-carbamoylethyl, and 4-aminobutyl. Presently preferred R_3 groups include tert-butyl, iso-butyl, benzyl, isopropyl and methyl.

R_4 may be, for example methyl or ethyl. Methyl is currently preferred.

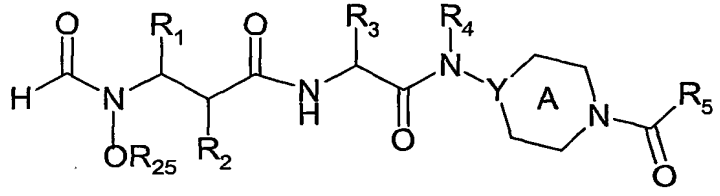
When m is 1, Alk^1 may be, for example, -(CH_2)- or -(CH_2CH_2)-.

Z may be, for example, a phenyl,pyridyl, thienyl, furanyl, pyranyl, pyrolyl, diazolyl, triazolyl, thiazolyl, thiadiazolyl, oxazolyl, ozadiazolyl, indolyl, benzisozazolyl, benzthiazolyl or imidazothiazolyl ring, optionally substituted as specified. Examples of optional substituents include methyl, methoxy, ethoxy, methoxymethyl, ethylthio, chloro, bromo, hydroxy, nitro, phenyl, 2- or 4-nitrophenyl, dimethylamino, dimethylaminophenyl, methylsulphonyl, dimethylaminosulphonyl, 3-pyridyl or 2-pyrazin-2-yl.

In one subgroup of the compounds of the invention, Z is a cyclopentyl, cyclohexyl, phenyl, morpholinyl, pyrimidin-2-yl, 1,2,3-thiadiazol-5-yl, 1,4-thiazol-5-yl, benzofuran-2-yl, 2-or 3-furanyl, 2- or 3-thienyl, 2- or 3-pyranyl, 2-, 3- or 4-pyrrolyl, 3-, 4- or 5-pyazolyl, 3-, 4- or 5-isoxazolyl, or 2-, 3- or 4-pyridyl ring any of which may optionally be substituted by hydroxy, methoxy, ethoxy, mercapto, methylthio, ethylthio, methyl, ethyl, trfluoromethyl, fluoro, chloro, amino, methylamino, or dimethylamino.

Examples of specific compounds of the invention are those of the Examples herein. . In those Examples, where a compound of formula (II) above wherein Q is an N-formylhydroxylamine radical -N(OH)CH(=O) is disclosed, it is to be understood that the equivalent compound wherein Q is a hydroxamate radical -C(=O)NH(OH) is also a specific compound of the invention, and *vice versa*.

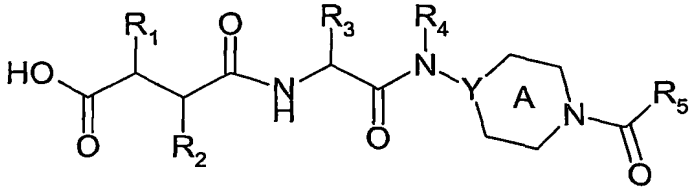
Compounds of the invention in which Q is an N-formylhydroxyamino group may be prepared by deprotecting an O-protected N-formyl-N-hydroxyamino compound of formula (III):



(III)

in which R_1, R_2, R_3, R_4, R_5 and Y are as defined in general formula (II) and R_{25} is a hydroxy protecting group removable to leave a hydroxy group by hydrogenolysis or hydrolysis. Benzyl is a preferred R_{25} group for removal by hydrogenolysis, and tert-butyl and tetrahydropyranyl are preferred groups for removal by acid hydrolysis.

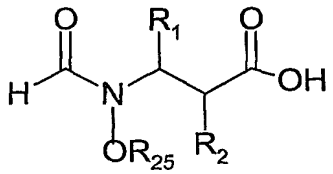
Compounds of the invention in which Q is a hydroxamic acid group may be prepared by reacting the parent compound wherein Q is a carboxylic acid group (IIIA)



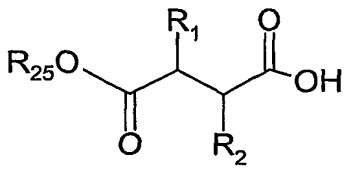
(IIIA)

with hydroxylamine or an N- and/or O-protected hydroxylamine, and thereafter removing any O- or N-protecting groups

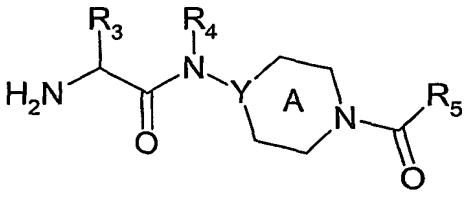
Compounds of formula (III) or (IIIA) may be prepared by causing an acid of formula (IV) or (IVA) or an activated derivative thereof to react with an amine of formula (V)



(IV)



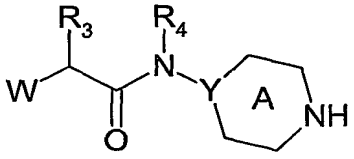
(IVA)



(V)

wherein R_1, R_2, R_3, R_4, R_5 and Y are as defined in general formula (II) except that any substituents in R_1 R_2, R_4, and R_5which are potentially reactive in the coupling reaction may themselves be protected from such reaction, and R_{25} is as defined in relation to formula (III) above, and optionally removing protecting groups R_1, R_2, R_4, and R_5.

Compounds of formula (V) may be prepared by N-acylation of a compound of formula (VI) with, for example, the acid chloride R_5COCl



(VI)

wherein R_4, R_5 and Y are as defined in general formula (V), and W is a protected amino group, and then removing the amino protecting group(s).

Compounds (II) of the invention may also be prepared by N-acylation of the corresponding piperazine or piperidine parent compound, as in the Examples herein.

Salts of the compounds of the invention include physiologically acceptable acid addition salts for example hydrochlorides, hydrobromides, sulphates, methane sulphonates, p-toluenesulphonates, phosphates, acetates, citrates, succinates, lactates, tartrates, fumarates and maleates. Salts may also be formed with bases,

for example sodium, potassium, magnesium, and calcium salts.

Compositions with which the invention is concerned may be prepared for administration by any route consistent with the pharmacokinetic properties of the active ingredient(s).

Orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

Safe and effective dosages for different classes of patient and for different disease states will be determined by clinical trial as is required in the art. It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body

weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

The following Examples illustrate embodiments of the invention. In the Examples, the following abbreviations have been used throughout:

DCM Dichloromethane
DIEA Diisopropylethylamine
DMF Dimethylformamide
HATU O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt 1-Hydroxy-7-benzotriazole
HPLC High performance liquid chromatography
LRMS Low resolution mass spectrometry
NMR Nuclear Magnetic Resonance
PyAOP7 Azabenzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
rt Room temperature
RT Retention time
TBTU 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluorophosphate
TFA Trifluoroacetic acid

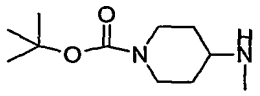
^{1}H and ^{13}C spectra were recorded using a Bruker DPX 250 spectrometer at 250.1 MHz (62.5 MHz for the ^{13}C). Chemical shift values are expressed in δ (ppm) and abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, m = multiplet, b = broad and app = apparent. Mass spectra were obtained using a Perkin Elmer Sciex API 165. Analytical HPLC was run on a Beckman System Gold, using Waters Symmetry C18 column (50 mm, 4.6 mm) with 20 to 90% solvent B gradient (1.5 ml/min) as the mobile phase. [Solvent A: 0.05%

TFA in 10% MeCN 90% water, Solvent B: 0.05% TFA in 10% water 90% MeCN, 5 min gradient time], detection wavelength at 220 or 214 nm. Preparative HPLC was run on a Gilson autoprep instrument using a C18 Waters delta pak (15μm, 300 Å, 25 mm, 100 mm) with 10 to 90% solvent B gradient as the mobile phase at a flow rate of 15 ml/min. [Solvent A 10% MeCN/water; Solvent B: 10% water/MeCN, 8 min gradient time], UV detection was at 220 or 214 nm.

Preparation of 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)propionylamino]-3,3,*N*-trimethyl-*N*-piperidin-4-yl-butyramide (Intermediate 1)

Step 1

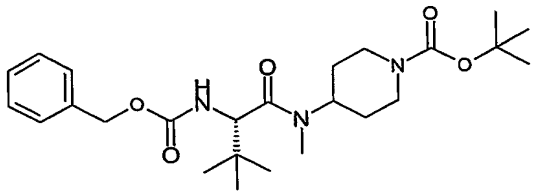
4-Methylamino-piperidine-1-carboxylic acid *tert*-butyl ester



To a solution of *tert*-butoxy-piperidone (10 g, 50 mmol) in EtOH (100 ml) was added N-methylamine (10 ml of a 33% solution in EtOH) and palladium on carbon (1 g, 10% w/w), hydrogen was bubbled through the reaction mixture for 2 h. The mixture was stirred under a blanket of hydrogen for 18 h at RT. The Pd/C was filtered off and the solvent removed *in vacuo* to yield the title compound as a clear oil. LRMS: +ve ion 215 (M+1, 20%), 237 (M+Na, 15%). ^{1}H-NMR (250MHz), δ ($CDCl_3$) 4.05 (2H, m), 2.80 (2H, m), 2.51 (1H, m), 2.45 (3H, s), 1.87 (2H, m), 1.62 (1H, s), 1.45 (9H, s), 1.25 (2H, m).

Step 2

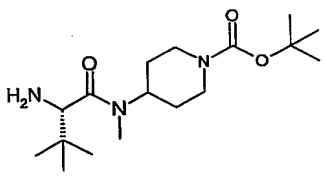
4-[(2-Benzyloxycarbonylamino-3,3-dimethyl-butyryl-methyl-amino]-piperidine-1-carboxylic acid *tert*-butyl ester



To a solution of Cbz-protected *tert*-leucine (500 mg, 1.9 mmol) in dichloromethane (10 ml) was added PyAOP (1.08 g, 2.1 mmol), HOAt (26 mg, 0.19 mmol), triethylamine and 4-Methylamino-piperidine-1-carboxylic acid *tert*-butyl ester (606 mg, 2.8 mmol). The reaction mixture was stirred for 18 h at Rt. The solvent was removed *in vacuo* and the yellow residue was redissolved in dichloromethane (80 ml) and was washed with 1M hydrochloric acid (2 x 80 ml), 1M sodium carbonate (2 x 80 ml), brine (1 x 80 ml) and dried over anhydrous magnesium sulphate to yield a clear oil (2 g). Flash chromatography (2% MeOH, dichloromethane) yielded the title compound as a white foam (870 mg, 100%) which contained a slight impurity. HPLC. 6.9 min (85%); LRMS: +ve ion 462 (M+1, 5%), 484 (M+Na, 20%). The compound was progressed to the next step without any further purification.

Step 3

4-[(2-Amino-3,3-dimethyl-butyryl)-methyl-amino]-piperidine-1-carboxylic acid *tert*-butyl ester

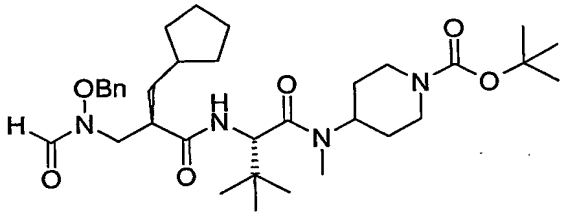


To a solution of 4-[(2-Benzyloxycarbonylamino-3,3-dimethyl-butyryl-methyl-amino]-

piperidine-1-carboxylic acid *tert*-butyl ester (500 mg, 1.1 mmol) in EtOH (10 ml) was added palladium on carbon (50 mg, 10% w/w), hydrogen was bubbled through the reaction mixture for 2 h. The mixture was stirred under a blanket of hydrogen for 18 h at Rt. The Pd/C was filtered off and the solvent removed *in vacuo* to yield the title compound as a yellow oil which was progressed to the next step without further purification. LRMS: +ve ion 328 (M+1, 100%).

Step 4

4-({2-[3-(Benzyloxy-formyl-amino)-2-cyclopentylmethyl-propionylamino]-3,3-dimethyl-butyryl}-methyl-amino)-piperidine-1-carboxylic acid *tert*-butyl ester

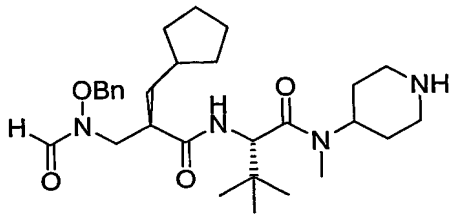


To a solution of of 3-(Benzyloxy-formyl-amino)-2*R*-cyclopentylmethyl-propionic acid (288 mg, 0.94 mmol) in dichloromethane/DMF, (4:1, 25 ml) was added WSC (198 mg, 1.04 mmol), HOAt (13 mg, 94.5 μmol), triethylamine (196 μl, 1.4 mmol) and 4-[(2-Amino-3,3-dimethyl-butyryl)-methyl-amino]-piperidine-1-carboxylic acid *tert*-butyl ester (340 mg, 1.04 mmol) at Rt. The reaction mixture was stirred for 18 h at Rt and the solvent was removed *in vacuo* to yield a yellow oil which was redissolved in dichloromethane (70 ml) and was washed with 1M hydrochloric acid (1 x 50 ml), 1M sodium carbonate (1 x 50 ml), brine (1 x 50 ml) and was dried over anhydrous magnesium sulphate. The solvent was removed under vacuum to yield a clear oil (517 mg). Flash chromatography gradient 2:1 to 1:1 hexane/ethyl acetate yielded the title compound as a white foam (300 mg, 52%). . HPLC. 7.1 min (85%); LRMS:

+ve ion 462 (M+1, 5%), 484 (M+Na, 20%)

Step 5

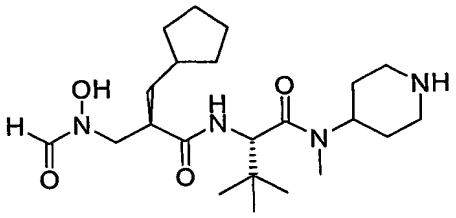
2-[3-(Benzyloxy-formyl-amino)-2-cyclopentylmethyl-propionylamino]-3,3,*N*-trimethyl-*N*-piperidin-4-yl-butyramide



To a solution of 4-({2-[3-(Benzyloxy-formyl-amino)-2-cyclopentylmethyl-propionylamino]-3,3-dimethyl-butyryl}-methyl-amino)-piperidine-1-carboxylic acid *tert*-butyl ester in dichloromethane (5 ml) was added acetic acid (1 ml) and then boron trifluoride etherate (91 µl, 0.71 mmol) at 0 °C. The reaction mixture was stirred at 0 C for 1.25 h, more dichloromthane was added (15 ml) and the organic layer was then washed with 1M sodium carbonate (1 x 30 ml), dried over anhydrous magnesium sulphate and the solvent was removed *in vacuo* to yield the title compound as a clear oil (98 mg, 78%). HPLC. 5.2 min (70%), salt formation due to secondary nitrogen is likely hence the purity; LRMS: +ve ion 515 (M+1, 100%). ^{1}H-NMR (250MHz), δ ($CDCl_3$) 8.14 (0.6 H, brs, 8.14), 7.87 (0.4H, brs, 7.87), 7.37-7.26 (5H, m), 6.29 (0.4H, d, J 9.4 Hz), 6.25 (0.6H, d, J 9.4 Hz), 5.01-4.71(3H, m), 4.55-4.48 (1H, m), 3.8 (2H, brm), 3.20-3.10 (3H, m), 2.99 (2H, s), 2.81 (1H, s), 2.74-2.63 (2H, m), 2.60-2.55 (1H, m), 1.81-1.37 (13H, m), 1.07-0.97 (2H, m), 0.95-0.92 (11H, m).

Step 6

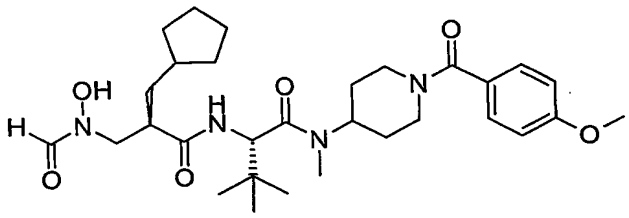
2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)propionylamino]-3,3,*N*-trimethyl-*N*-piperidin-4-yl-butyramide (Intermediate 1)



To a solution of 2-[3-(Benzyloxy-formyl-amino)-2-cyclopentylmethyl-propionylamino]-3,3,*N*-trimethyl-*N*-piperidin-4-yl-butyramide (135 mg, 0.26 mmol) in EtOH (5 ml) was added Pd/C (14 mg, 15% w/w), hydrogen was bubbled through the suspensioin for 2 h and the reaction was then stirred for 18 h at Rt under a blanket of hydrogen. The catalyst was filtered off and the solvent was removed *in vacuo* to yield the title compound as a white solid (98 mg, 88%). ; LRMS: +ve ion 425 (M+1, 100%), -ve ion 423 (M-1, 100%). The NMR was complex due to the presence of rotamers and a zwitterion.

Example 1

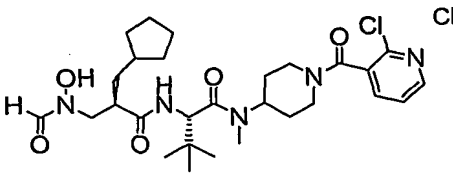
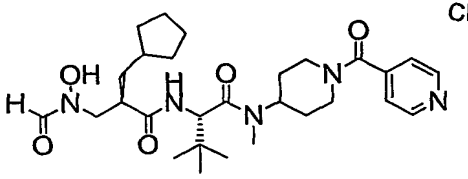
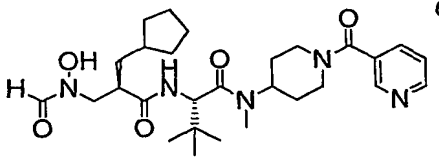
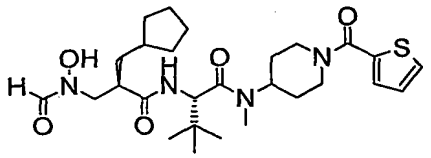
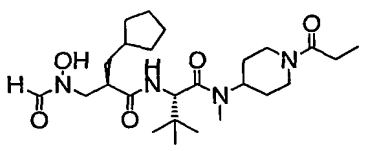
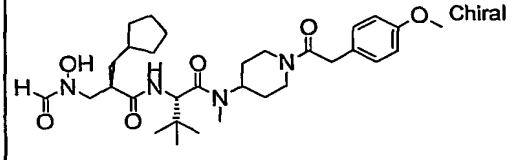
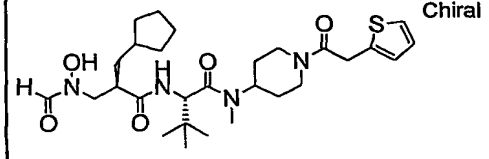
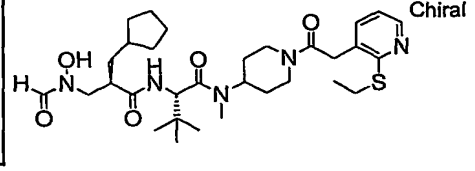
2-[2-Cyclopentyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(4-methoxy-benzoyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide

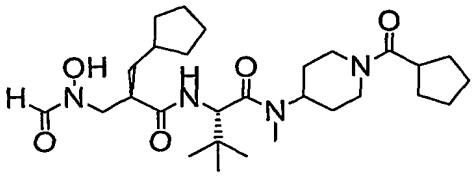
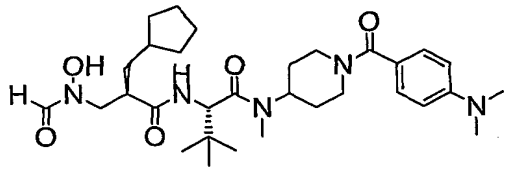
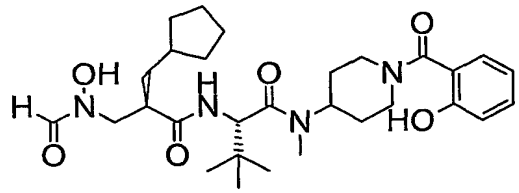


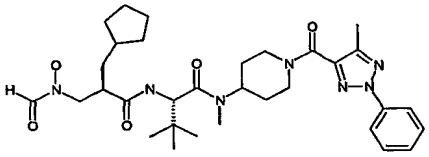
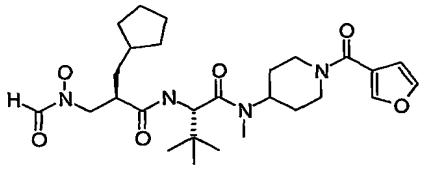
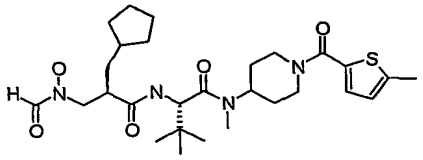
To a solution of 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)propionylamino]-3,3,*N*-trimethyl-*N*-piperidin-4-yl-butyramide (Intermediate 1) (150 mg, 0.4 mmol) in dichloromethane (5 ml) was added 4-methoxy-benzoyl chloride (90.5 mg, 0.5 mmol) and triethylamine (73.4 µl, 0.5 mmol). The reaction mixture was stirred at RT for 18h. Aminomethyl polystyrene resin (0.89 mmol/g, 3 eq excess) was added and the reaction mixture was left to stir for a further 18 h. The resin was filtered off and the solvent was removed *in vacuo*, to yield the crude product. The title compound was purified by preparative HPLC to yield a white solid. HPLC: RT: 5.1 min (82% @ 214nm); ESMS: +ve: 559 (M+1, 20%);
-ve: 557 (M-1, 40%).

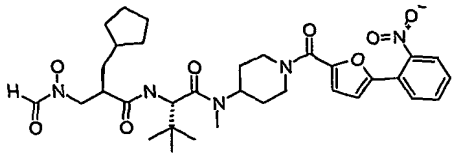
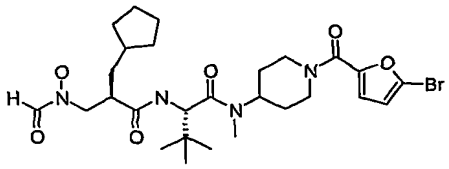
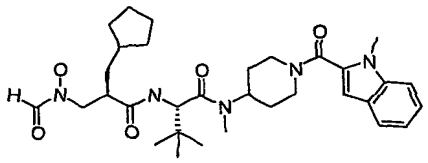
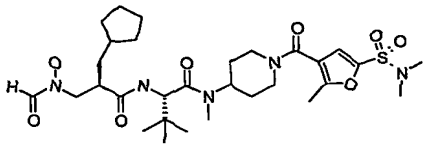
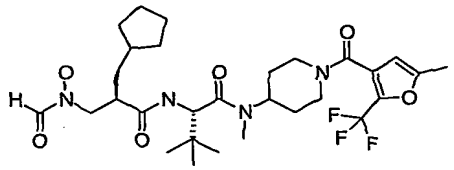
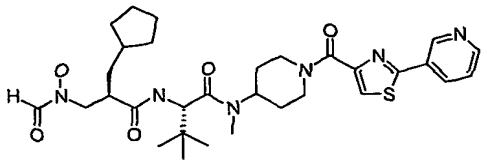
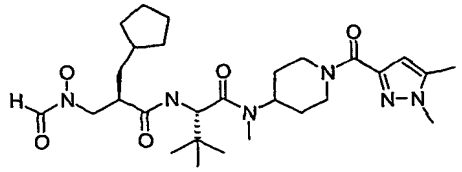
The minimum inhibitory concentrations of the compound of Example 1 was determined by the protocol described in the Antibacterial Assay section below. The range of MICs determined for the *Strep. pneumoniae* panel was 0.25-0.5 ngm/litre

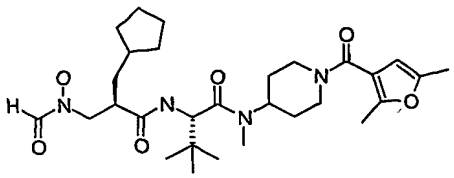
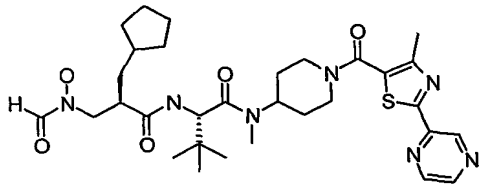
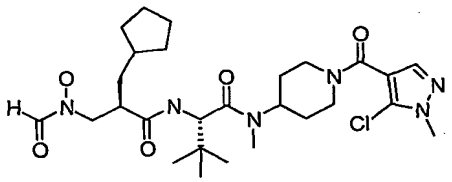
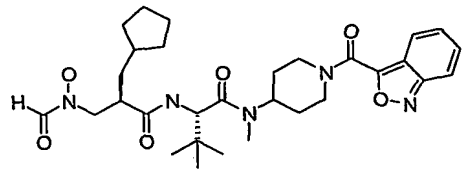
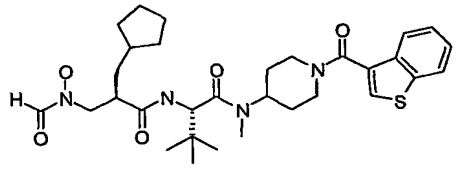
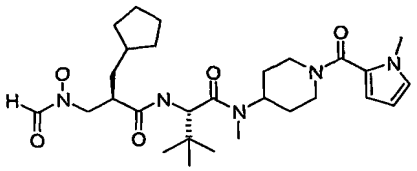
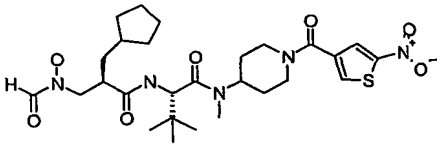
Compounds of Examples 2-56 were prepared in a manner analogous to that of Example 1. All compounds were purified by preparative HPLC

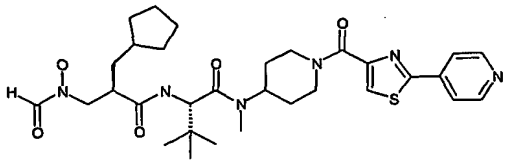
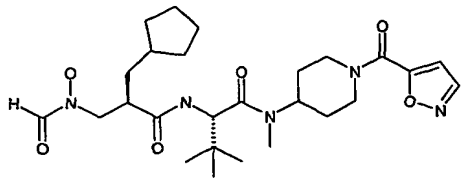
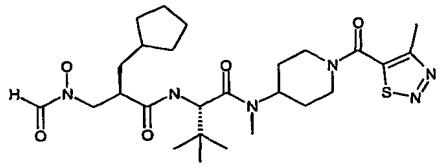
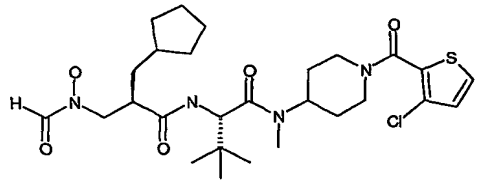
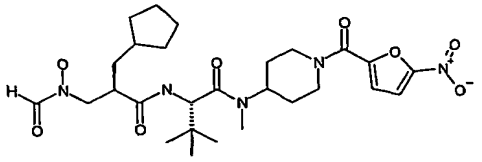
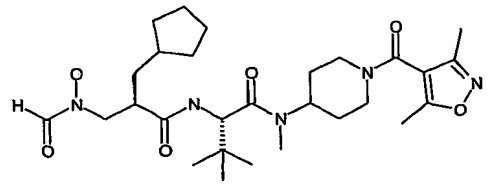
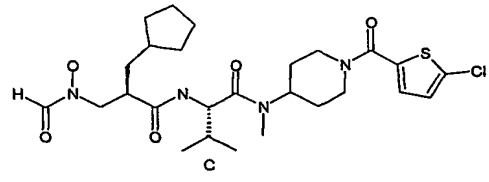
Compound No.	Structure	Analytical Data
2	 Chiral	HPLC : RT: 4.7 min (81% @ 214nm) ESMS: +ve: 564 (M+1, 30%); -ve: 563 (M-1, 10%)
3	 Chiral	HPLC: RT: 1.8 min (89% @ 214 nm) ESMS:+ve: 530 (M+1, 60%), 552 (M+Na, 5%); -ve 528 (M-1, 10%)
4	 Chiral	HPLC: 'RT: 2.0 min (85% @ 214 nm) ESMS: +ve: 530 (M+1, 100%); -ve: 528 (M-1, 20%)
5	 Chiral	RT: 5.1 min (80% @ 214 nm) ESMS: +ve: 535 (M+1, 5%); -ve: 533(M-1, 30%)
6	 Chiral	HPLC: RT: 4.8 min (76%) @ 214 nm ESMS: +ve: 481(M+1), 503 (M+Na); -ve: 479 (M-1)
7	 Chiral	HPLC: RT: 5.3 min (100%) ESMS: +ve: 573(M+1), 595 (M+Na); -ve: 571 (M-1)
8	 Chiral	HPLC: RT: 5.2 min (100%) @ 214 nm ESMS: +ve: 549 (M+1), 571 (M+Na); -ve: 547 (M-1)
9	 Chiral	HPLC: RT: 5.3 min (99%) @ 214 nm ESMS: +ve: 590 (M+1), 612 (M+Na); -ve: 588 (M-1)

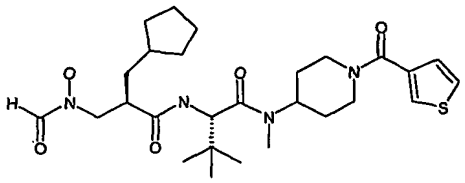
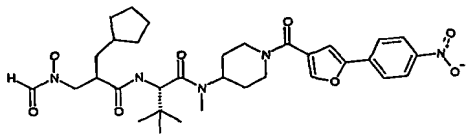
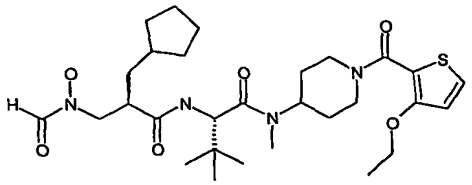
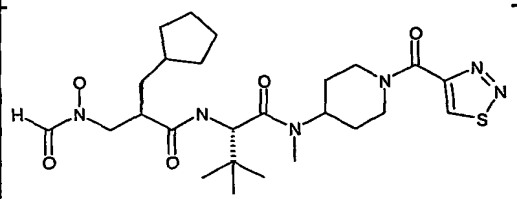
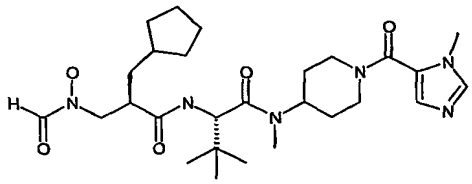
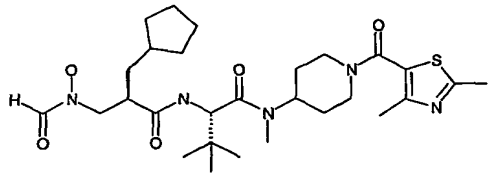
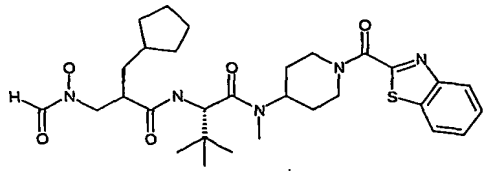
10	 Chiral	HPLC: RT: 5.3 min (100%) @ 214 nm ESMS: +ve: 521 (M+1), 543 (M+Na); -ve: 519 (M-1)
11	 Chiral	HPLC: RT: 4.8 min (100%) @ 214 nm ESMS: +ve: 572 (M+1), 594 (M+Na)
12	 Chiral	HPLC: RT: 5.2 min (92%) @214 nm ESMS: +ve: 567 (M+Na), 545 (M+1); - ve: 543 (M-1)

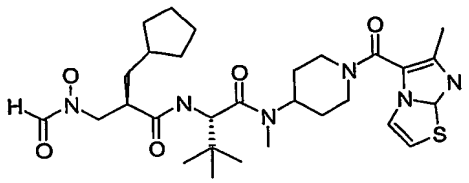
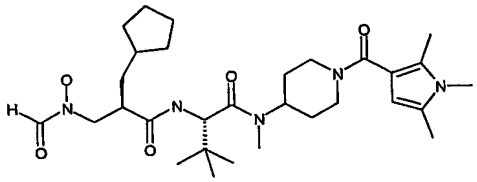
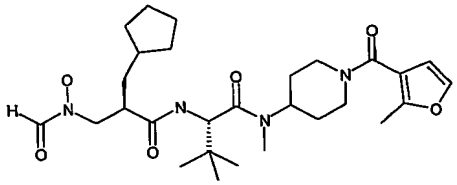
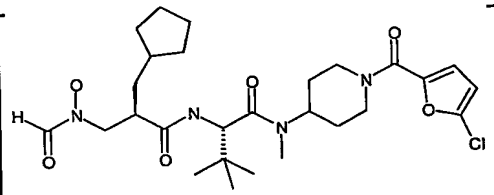
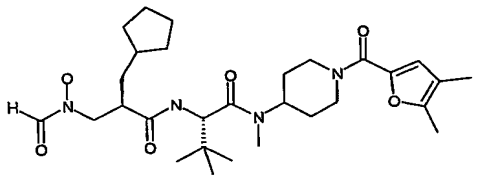
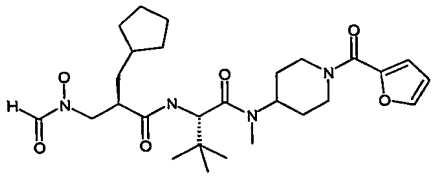
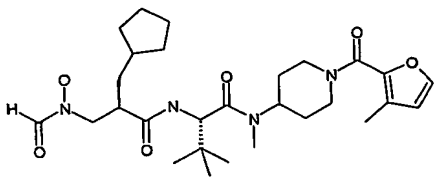
Compound no	Structure	ANALYTICAL METHOD	ANALYTICAL DATA
13		HPLC ESMS	RT 5.5 min (99% @ 214 nm) +ve: 610 (M+1, 80%) -ve: 608 (M-1, 100%)
14		ESMS HPLC	+ve: 519(M+1), 541 (M+Na) -ve: 517(M-1) RT: 4.5 min (99%)
15		ESMS HPLC	+ve: 549 (M+1), 571(M+Na) -ve: 547(M-1) RT: 5.1 min (99%)

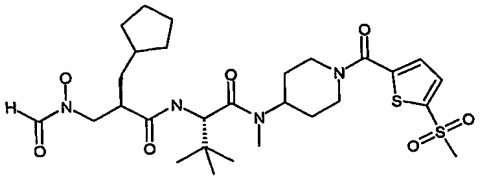
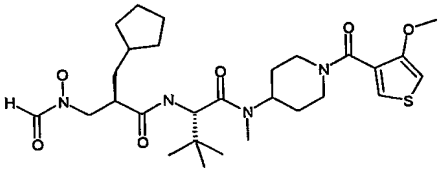
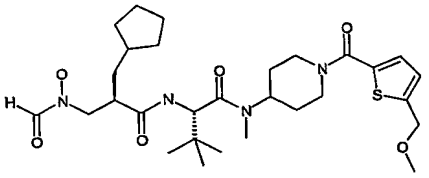
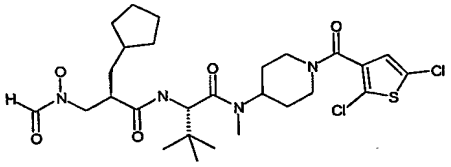
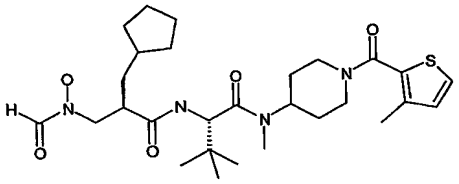
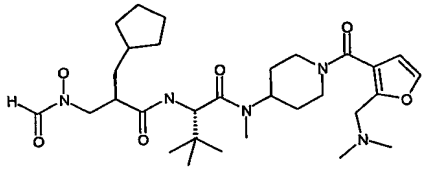
16		ESMS HPLC	+ve: 640(M+1), 662 (M+Na) -ve: 638(M-1) RT: 5.3 min (100%)
17		ESMS HPLC	+ve: 598 (M+1), 620 (M+Na) -ve: 596 (M-1) RT: 5.0 min (99%)
18		ESMS HPLC	+ve: 582 (M+a), 604 (M+Na) -ve: 580 (M-1) RT: 5.4 min (99%)
19		ESMS HPLC	+ve: 640 (M+1), 662(M+Na) -ve: 638(M-1) RT: 4.9 (99%)
20		ESMS HPLC	+ve: 601(M+1), 623 (M+Na) -ve: 599 (M-1) RT: 5.3 min (99%)
21		ESMS HPLC	+ve: 613(M+1), 635 (M+Na) -ve: 611(M-1) RT: 3.2 min (99%)
22		ESMS HPLC	+ve: 547 (M+1), 569 (M+Na) -ve: 545 (M-1) RT: 4.3 min (100%)

23		ESMS HPLC	+ve: 547 (M+1), 569 (M+Na) -ve: 545(M-1) RT: 5.0 min (99%)
24		ESMS HPLC	+ve: 628 (M+1), 650 (M+Na) -ve: 626(M-1) RT: 4.7 min (100%)
25		ESMS HPLC	+ve: 567 (M+1), 589 (M+Na) -ve: 565 (M-1) RT: 4.3 min (99%)
26		ESMS HPLC	+ve: 570(M+1), 592 (M+Na) -ve: 568(M-1) RT: 5.1 min (99%)
27		ESMS HPLC	+ve: 585 (M+1), 607(M+Na) -ve: 583(M-1) RT: 5.4 min (99%)
28		ESMS HPLC	+ve: 532(M+1), 554(M+Na) -ve: 530(M-1) RT: 4.8 min (99%)
29		ESMS HPLC	+ve: 580 (M+1), 602 (M+Na) -ve: 578 (M-1) RT: 4.9 min (96% @214 nm)

30		HPLC ESMS	RT: 1.6 min (100% @214 nm) +ve: 613 (M+1), 635 (M+Na) -ve: 611 (M-1)
31		HPLC ESMS	RT: 4.3 min (99% @ 214 nm) +ve: 520 (M+1), 542 (M+Na) -ve: 518 (M-1)
32		ESMS HPLC	+ve: 551 (M+1), 573 (M+Na) -ve: 549 (M-1) RT: 4.5 min (99%)
33		HPLC ESMS	RT: 5.0 min (98% @ 214 nm) +ve: 569 (M+1), 591 (M+Na) -ve: 567 (M-1)
34		HPLC ESMS	RT: 4.8 min (90% @ 214 nm) +ve: 564 (M+1), 586 (M+Na) -ve: 562 (M-1)
35		ESMS HPLC	+ve: 548 (M+1), 570 (M+Na) -ve: 546 (M-1) RT: 4.4 min (98% @ 214 nm)
36		HPLC ESMS	RT: 5.3 min (99% @ 214 nm) +ve: 569 (M+1), 591 (M+Na) -ve: 567 (M-1)

37		HPLC ESMS	RT: 4.6 min (99% @ 214 nm) +ve: 640 (M+1), 662 (M+Na) -ve: 638 (M-1)
38		HPLC ESMS	RT: 5.4 min (97% @ 214 nm) +ve: 640 (M+1), 662 (M+Na) -ve: 638 (M-1)
39		HPLC ESMS	RT: 5.0 min (99% @ 214 nm) +ve: 579 (M+1), 601 (M+Na) -ve: 577 (M-1)
40		ESMS HPLC	+ve: 537 (M+1, 559 (M+Na) -ve: 535 (M-1) RT: 4.2 min (99% @ 214 nm)
41		HPLC ESMS	RT: 5.0 min (96% @ 214 nm) +ve: 533 (M+1), 555 (M+Na)
42		HPLC ESMS	RT: 4.0 min (98% @ 214 nm) +ve: 564(M+1), 586 (M+Na) -ve: 562 (M-1)
43		HPLC ESMS	RT: 5.4 min (>95% @ 214 nm) +ve: 586 (M+1), 611 (M+Na) -ve: 584 (M-1)

44		ESMS HPLC	+ ve: 589 (M+1) RT: 4.5 min (>90%)
45		ESMS HPLC	+ive: 560, 45%, M+1; 582, 5%, M+Na. 214nm, 5.20 min, 94%.
46		ESMS HPLC	+ive: 533, 30%, M+1; 555, 100%, M+Na. 214nm, 4.84 min, 100%
47		ESMS HPLC	+ive: 553, 20%, M+1; 575, 100%, M+Na. 214nm, 5.14 min, 100%
48		ESMS HPLC	+ive: 547, 30%, M+1; 569, 100%, M+Na 214nm, 5.11 min, 99%
49		ESMS HPLC	+ive: 519, 10%, M+1; 541, 100%, M+Na 214nm, 4.71 min, 100%
50		ESMS HPLC	+ive: 533, 50%, M+1; 555, 100%,M+Na 214nm, 4.94 min, 98%.

51		ESMS HPLC	+ive: 613, 15%, M+1; 635, 100%, M+Na 214nm, 4.75 mins, 100%
52		ESMS HPLC	+ive: 565, 20%, M+1; 587, 100%, M+Na 214nm, 4.84 min, 100%
53		ESMS HPLC	+ive: 579, 20%, M+1; 601, 100%, M+Na 214nm, 4.92 min, 100%
54		ESMS HPLC	+ive: 603, 15%, M+1; 625, 100%, M+Na 214nm, 5.61 min, 100%
55		ESMS HPLC	+ive: 549, 35%, M+1; 571, 100%, M+Na 214nm, 5.04 min, 100%
56		ESMS HPLC	+ive:576, 100%, M+1, 598, 15%, M+Na -ive: 574, 100%, M-1 214nm, 4.38 min, 100%

The minimum inhibitory concentrations of compounds 2 to 56 were also determined by the protocol described in the Antibacterial Assay section below. In each case the range of MICs determined for the *Strep. pneumoniae* panel is given below, alongside the compound name:

Compound 2: *N*-[1-(2-Chloro-pyridine-3-carbonyl)-piperidin-4-yl]-2-[2--cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide. MIC 0.25-0.50

Compound 3: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(pyridine-4-carbonyl)-piperidin-4-yl]-butyramide. MIC <0.125-0.25

Compound 4: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(pyridine-3-carbonyl)-piperidin-4-yl]-butyramide. MIC 1.0-2.0

Compound 5: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(thiophene-2-carbonyl)-piperidin-4-yl]-butyramide. MIC 0.25-0.50

Compound 6: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-propionyl-piperidin-4-yl]-butyramide. MIC 1.0-2.0

Compound 7: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-{1-[2-(4-methoxy-phenyl)-acetyl]-piperidin-4-yl}-3,3,*N*-trimethyl-butyramide. MIC 0.25

Compound 8: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(2-thiophen-2-yl-acetyl)-piperidin-4-yl]-butyramide. MIC 0.25

Compound 9: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-{1-[2-(2-ehtylsulfanyl-pyridin-3-yl)acetyl]-piperidin-4-yl}-3,3,*N*-trimethyl-butyramide.

MIC 0.125-0.25

Compound 10: *N*-(1-Cyclopentanecarbonyl-piperidin-4-yl)-2[2-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide. MIC 0.25-0.50

Compound 11: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(4-dimethylamino-benzoyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide. MIC <0.125-0.25

Compound 12: 2-[2-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(2-hydroxy-benzoyl)-piperidin-4-yl]-3,3, *N*-trimethyl-butyramide. MIC <0.125

Compound 13 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(5-methyl-2-phenyl-2*H*-[1,2,3]triazole-4-carbonyl)-piperidin-4-yl]-butyramide. MIC MIC <0.125-0.25

Compound 14 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(furan-3-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide. MIC <0.125-0.5

Compound 15 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(5-methyl-thiophene-2-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 16 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-{1-[5-(2-nitro-phenyl)-furan-2-carbonyl]-piperidin-4-yl}-butyramide MIC <0.125

Compound 17 *N*-[1-(5-Bromo-furan-2-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.5

Compound 18 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(1-methyl-1*H*-indole-2-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 19 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(5-dimethylsulfamoyl-2-methyl-furan-3-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.25

Compound 20 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(5-methyl-2-trifluoromethyl-furan-3-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 21 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(2-pyridin-3-yl-thiazole-4-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 22 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(1,5-dimethyl-1*H*-pyrazole-3-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.5

Compound 23 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(2,5-dimethyl-furan-3-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.25

Compound 24 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(4-methyl-2-pyrazin-2-yl-thiazole-5-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 25 *N*-[1-(5-Chloro-1-methyl-1*H*-pyrazole-4-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-

butyramide MIC <0.125-0.5

Compound 26 *N*-[1-(Benzo[c]isoxazole-3-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC <0.1250.25

Compound 27 *N*-[1-(Benzo[b]thiophene-3-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC <0.1250.25

Compound 28 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(1-methyl-1*H*-pyrrole-2-carbonyl)-piperidin-4-yl]-butyramide MIC <0.5-1.0

Compound 29 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(5-nitro-thiophene-3-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125-0.25

Compound 30 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(2-pyridin-4-yl-thiazole-4-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125-0.25

Compound 31 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(isoxazole-5-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.5

Compound 32 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(4-methyl-[1,2,3]thiadiazole-5-carbonyl)-piperidin-4-yl]-butyramide MIC <0.25-0.5

Compound 33 *N*-[1-(3-Chloro-thiophene-2-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-

cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.5

Compound 34 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(5-nitro-furan-2-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 35 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(3,5-dimethyl-isoxazole-4-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.125

Compound 36 *N*-[1-(5-Chloro-thiophene-2-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC <0.125

Compound 37 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(thiophene-3-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125-0.5

Compound 38 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-{1-[5-(4-nitro-phenyl)-furan-3-carbonyl]-piperidin-4-yl}-butyramide MIC <0.125-0.25

Compound 39 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(3-ethoxy-thiophene-2-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.25

Compound 40 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-([1,2,3]thiadiazole-4-carbonyl)-piperidin-4-yl]-butyramide MIC <0.125

Compound 41 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3-dimethyl-*N*-[1-(3-methyl-3*H*-imidazole-4-carbonyl)-piperidin-4-yl]-*N*-methylbutyramide MIC <0.125-0.25

Compound 42 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(2,4-dimethyl-thiazole-5-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.5-1.0

Compound 43 *N*-[1-(Benzothiazole-2-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC <0.125-0.25

Compound 44 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(6-methyl-7,7a-dihydro-imidazo[2,1-b]thiazole-5-carbonyl)-piperidin-4-yl]-butyramide MIC 1.0-4.0-

Compound 45 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-*N*-[1-(1,2,5-trimethyl-1*H*-pyrrole-3-carbonyl)-piperidin-4-yl]-butyramide MIC 4.0-8.0

Compound 46 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3-dimethyl-*N*-[1-(2-methyl-furan-3-carbonyl)-piperidin-4-yl]-*N*-methylbutyramide MIC 0.06-0.5

Compoun 47 *N*-[1-(5-Chloro-furan-2-carbonyl)-piperidin-4-yl]-2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3,*N*-trimethyl-butyramide MIC 0.06-0.5

Compound 48 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(4,5-dimethyl-furan-2-carbonyl)piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC 0.06-0.5

Compound 49 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(furan-2-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC 0.125-1.0

Compound 50 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3-dimethyl-*N*-[1-(3-methyl-furan-2-carbonyl)-piperidin-4-yl]-*N*-methylbutyramide MIC 0.06-1.0

Compound 51 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(5-methanesulphonyl-thiophene-2-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC 0.125-0.5

Compound 52 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(4-methoxyl-thiophene-3-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC 0.125-0.25

Compound 53 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(5-methoxymethyl-thiophene-2-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC 0.125-0.5

Compound 54 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(2,5-dichloro-thiophene-2-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC <0.03-0.5

Compound 55 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-3,3-dimethyl-*N*-[1-(3-methyl-thiophene-2-carbonyl)-piperidin-4-yl]-*N*-methylbutyramide MIC 0.125-0.5

Compound 56 2*S*-[2*R*-Cyclopentylmethyl-3-(formyl-hydroxy-amino)-propionylamino]-*N*-[1-(2-dimethylaminomethyl-furan-3-carbonyl)-piperidin-4-yl]-3,3,*N*-trimethyl-butyramide MIC 0.25-1.0

Antibacterial Assay

Minimal Inhibitory concentrations (MIC) of inhibitors against clinical isolates of *S. pneumoniae*, obtained from the Public Health and Clinical Microbiology Laboratory, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QW, UK, were determined by a standard agar plate dilution method following recommendations in **British Society for Antimicrobial Chemotherapy Working Party**. 1991. "A guide to sensitivity testing British Society for Antimicrobial Chemotherapy, London, United Kingdom". Briefly Iso-Sensitest agar (pH 7.2: Oxoid, United Kingdom) was employed supplemented with 5% horse blood (Oxoid) and 20 μg of NAD (Sigma) per ml to support growth of fastidious bacteria. The inoculum used was approximately 10^4 colony forming units of each isolate contained in a volume of 1 μl. Plates were incubated 18 to 24 hr in air, or for fastidious bacteria an atmosphere enriched with 4-6% carbon dioxide at 35°C. The MIC was determined as the lowest concentration of an antimicrobial tested that inhibited growth of the inoculum, disregarding a single persisting colony or faint haze caused by the inoculation. MICs were expressed as ngm/litre.